STATOLITH FORMATION IN CNIDARIA: EFFECTS OF CADMIUM ON AURELIA STATOLITHS

D. B. Spangenberg

Department of Pathology
Eastern Virginia Medical School
Norfolk, VA 23501
Phone No: (804) 446-5626

Abstract

Statolith formation in Cnidaria was reviewed with an emphasis on Aurelia statoliths. The review provides information on the chemical composition, mechanisms of initiation of mineralization, and effects of environmental factors on Cnidarian statolith formation. Environmental factors discussed include modified sea water ingredients, X-irradiation, clinostat rotation, and petroleum oil ingredients. A detailed account of the effects of cadmium on mineralization and demineralization of Aurelia statoliths is given. Cadmium at dosages of 2 to 4 μM significantly reduces statolith numbers in developing ephyrae. At a dosage of 3 μM, cadmium accelerates statolith loss in unfed ephyrae studied at 4 and 8 days following ephyra release from strobilae. Cadmium, therefore, is shown to reduce statolith numbers in developing ephyrae and to cause greater reduction of statolith numbers in unfed ephyrae after 4 and 8 days than occurred in controls. Supplementation of Cd^{2+}-containing artificial sea water (ASW) with calcium (3X and 5X ASW calcium content) results in higher numbers of statoliths at day 4 as compared with cadmium-treated ephyrae. At 8 days only the 5X calcium supplemented ASW is effective in enhancing statolith numbers in Cd^{2+}-treated ephyrae. These results suggest that cadmium competes in some manner with calcium at the mineralizing sites of Aurelia.

KEY WORDS: Jellyfish, Aurelia, metamorphosis, statoliths, mineralization, demineralization, calcium sulfate, ephyra, calcifying vesicles.

Introduction

In Cnidaria, medusae of Hydrozoa and Scyphozoa form statocysts consisting of lithocytes with intracellular statoliths. Anthozoans and some primitive leptomedusae lack statocysts and statoliths (Hyman, 1940; Chapman, 1985). Singla (1975), in his detailed account of the statocysts of Hydromedusae describes statocysts of Leptomedusae, Mitrocomella polydiademata, Phialidium gregarium, Halistaura cellularia, Tiaropsis multicirrata, Aequorea aequorea, and two narcomedusae, Aegina citrea and Solmissus marshalli. He pointed out that some of the leptomedusae had open type statocysts (Mitrocomella) while others (Phialidium and Aequorea) are closed. Scyphozoans (including Aurelia aurita) have sensory clubs called rhopalia with statoliths at their distal ends. While considerable information is available concerning the morphology of the various types of statocysts in the different jellyfish medusae, only a few organisms have been studied with regard to the formation of their statoliths and the types of environmental factors which affect their development. This paper addresses the formation of statoliths, their chemical composition, and the environmental factors which have been studied in Aurelia which synthesize their statoliths during ephyra formation.

Chemical Composition of Statoliths

Medusae of Hydrozoa and Scyphozoa form statocysts consisting of statoliths which are situated at the tip of rhopalia (Schyphozoa) or are found in epidermal pits (Hydrozoa) or embedded in the bell margin. In some Cnidaria (Trachylina), statoliths are derived from gastrodermal cells, whereas in others (Leptomedusae) they are formed by epidermal cells. In Aurelia scyphomedusae, minute calcifying vesicles with tiny statoliths are found in cells in the epidermis, whereas cells with large fully-formed statoliths collect in the gastrodermis (Spangenberg, 1976).

Hyman (1940) reported that the statoliths of the Hydromedusae are composed of an organic material and calcium carbonate while the statoliths of Semaeostomes and Rhizostomes are composed of calcium sulfate dihydrate (gypsum) with a small admixture of calcium phosphate. Spangenberg and Beck (1968), using X-ray diffraction, X-ray spectrometry and polarizing microscopy, found that the statoliths of laboratory-

grown medusae contained only calcium sulfate dihydrate with no trace of calcium phosphates, carbonates, or oxalates. More recently, using X-ray microanalysis, Chapman (1985) verified that another scyphozoan jellyfish, Chrysaora hysoscella, has statoliths of calcium sulfate dihydrate, as do the cubozoans, Chiropsalmus, which he examined using X-ray microanalysis. Chapman, further, studied the statoliths of six leptomedusan families (Mitrocomella brownei, Phialella quadrata, Helgicirrha schulzei, Eutima gracilis, Phialidium hemisphaericum, and Lovenella clausa), and found that these organisms have statoliths composed of $MgCaPO_4$, in which Mg^{2+} is more abundant than Ca^{2+}. A trachymedusa, Aglantha digitale also had statoliths of $MgCaPO_4$, but their magnesium content was less abundant. These results do not support the statement of Hyman (1940) who reported that the statoliths of leptomedusae are composed of calcium carbonate plus organic matter or the report of Singla (1975) that some leptomedusae have concretions which contain calcium sulfate. Singla did, however, report that statoliths of Mitrocomella, Phialidium, and Aequorea probably contain calcium phosphate.

More recently, Spangenberg examined the statoliths of ephyrae from polyps of Aurelia (Fig. 1) collected at: Wood's Hole, Massachusetts; Norfolk, Virginia; Delaware; and the Thames, England, (Figure 2) using energy dispersive X-ray analysis. In all of these cases, calcium and sulfur but not phosphorus were found in the statoliths. Chapman (1985) reported a small peak of phosphorus associated with the statoliths of Aurelia and Chrysaora hysoscella which also had high peaks of calcium and sulfur.

Rhopalium and statolith development

The Cnidarian investigated in most detail in recent times with regard to rhopalium and statolith formation is Aurelia aurita. During strobilation, Aurelia polyps segment just beneath the oral region, giving rise to new segments approximately every 24 h until most of the organism is segmented (Figure 1). Spangenberg and Kuenning (1976) depicted segment formation and metamorphosis of the distal segment of Texas Aurelia using scanning electron microscopy (SEM). Although rhopalia develop at the bases of the primary tentacles of the polyps, they are not modified tentacles, but new structures. The observation that mineralization begins at the base of a tentacle (the upper portion of which ultimately degenerates) was earlier reported by Bigelow (1910) in Cassiopea xamachana during monodisk strobilation. In the Texas Aurelia, tiny statoliths were found at the base of the tentacles 10 h after the first segment formed and approximately 58 h after the organisms were induced to metamorphose at 27°C with iodine. The statoliths increase in number and size as the rhopalia develop so that the mature ephyrae contain usually eight rhopalia with 8 to 20 statoliths per rhopalium (Higher numbers have been subsequently found in the Norfolk Aurelia). The statoliths continue to increase in number and size as the ephyrae grow into medusae, resulting in several hundred statoliths per rhopalium in the adult medusae. That statolith formation is an in situ event was demonstrated by Spangenberg (1968) when the arm primordia were dissected from non-terminal strobila segments and maintained for 48 h in artificial sea water (ASW). Statoliths formed in 35.7% of the arm primordia. Many of the primordia, however, failed to form, due to tissue deterioration.

Initiation of Statolith Formation

Spangenberg (1976) used transmission electron microscopy (TEM) to detect minute statolith "ghosts" which represent the earliest signs of mineralization in ephyrae. These statolith "ghosts" are minute statoliths demineralized by the fixatives, glutaraldehyde and osmium. The minute statolith "ghosts" are found in calcifying vesicles (CV) inside cells which are located primarily in the epidermis. The CVs are membrane-bound and measure 1 to 5 μm in length or width. Some of the CVs contain osmiophilic material and internal membranous materials which are often concentric. Often, the vesicles are found near the cell nuclei and a Golgi apparatus is found closely associated with them. Of particular interest is the fact that CVs are acid phosphatase but not alkaline phosphatase positive.

The cells in which mineralization begins through CV formation have an irregularly shaped nucleus, Golgi apparatus, mitochondria, rough endoplasmic reticulum (RER), smooth endoplasmic reticulum (SER), occasional glycogen particles, and lipid vesicles. These cells are often vacuolated, and, some of them, when situated at the epidermal surface, reveal flagellae which are surrounded by microvilli. In addition, small vesicles resembling neurosecretory vesicles were seen in these calcifying cells.

Singla (1975) described the mineralizing cells of Aegina and Solmissus which are Narcomedusae. Most of the concretion material was lost during fixation except for membranous or fibrous material. The cell nucleus is triangular (Aegina) or bean-shaped (Solmissus). The cytoplasm contains a few mitochondria, a Golgi complex and a number of small vesicles.

The finding that cells in the epidermis of Aurelia form CVs which mineralize to produce minute statoliths is not consistent with the statement by Hyman (1940) that statoliths of scyphomedusae form in the gastrodermis. However, since the large numbers of mature statoliths of the statocyst do occur in the gastrodermis of the ephyrae, it is presumed that cells with the tiny statoliths are capable of migrating into the gastrodermis where they collect at the tip of the rhopalium and their statoliths grow. The fact that the smallest statoliths are usually found at the proximal end of the statocyst suggests that new statoliths are added to the statocyst in a regulated manner by the migration of cells with tiny intracellular statoliths.

Statolith Growth. Viewed with the TEM, mature intracellular statoliths of ephyrae, found only in the gastrodermis of the statocyst, are

Figure 2. Developmental stages of jellyfish: → Fig. 2A - polyp; Fig. 2B - early strobila; Fig. 2C - ephyra. Bar = 0.25 mm. (from Spangenberg, 1981, reproduced with permission of SEM, Inc.)

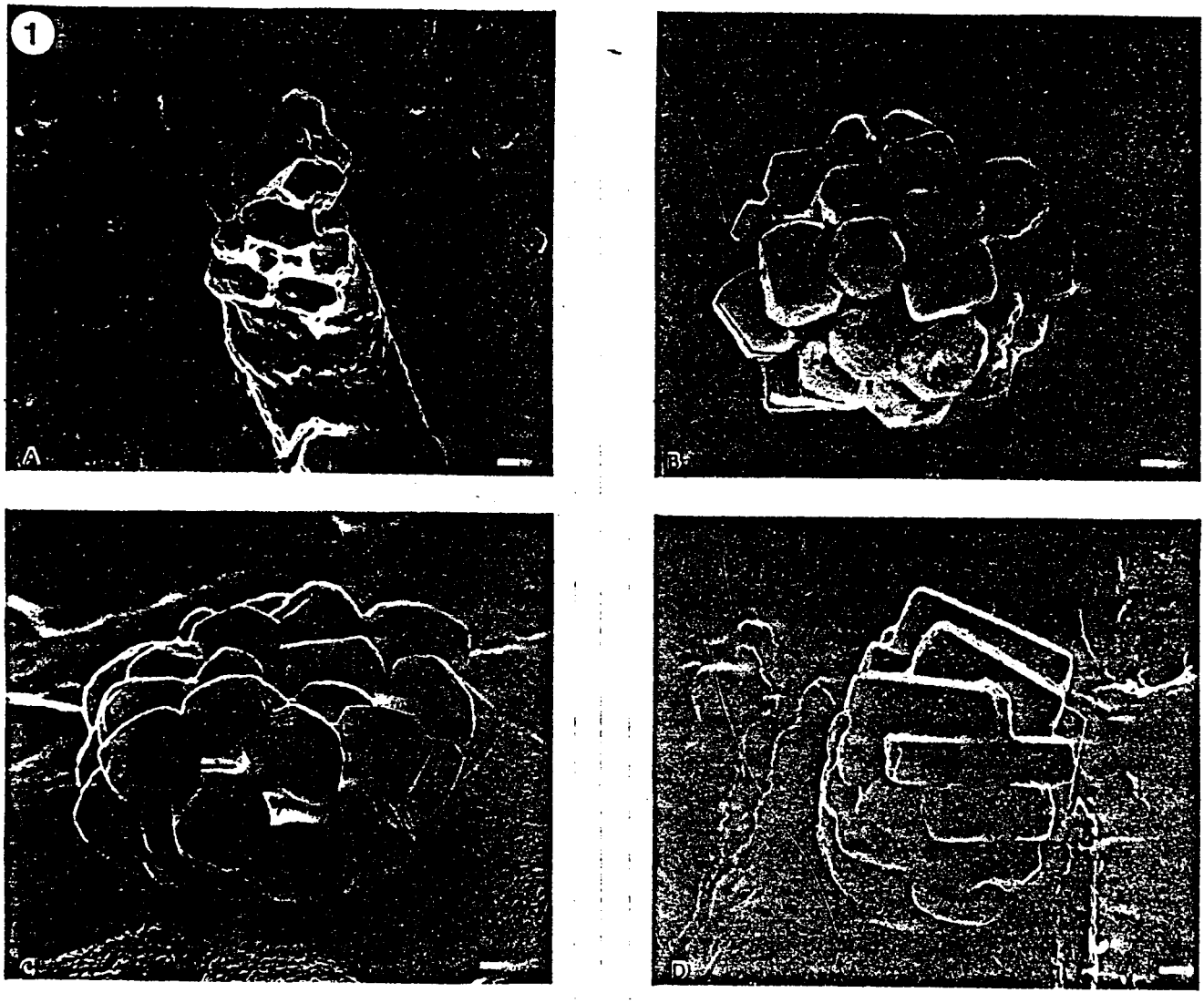

Figure 1. SEM of statoliths in statocysts at the tips of rhopalia of: A. Texas; B. Puerto Rican Aurelia; C. Woods Hole Aurelia; and D. Lambert's Aurelia from the Thames, England. Bar = 5 µm.

considerably longer (20-50 μM) than those in the CVs of cells in the epidermis. The mature statoliths in the lithocytes are sheath-bound and are contained within a large intracellular vacuole. Also found in many of the statolith-containing cells are a Golgi apparatus, mitochondria, ribosomes, and RER. Only one mature statolith is seen per cell. The fact that the mature statoliths are surrounded by a large vacuole indicates that, once mineralization has been initiated, the CV may grow into vacuoles which may facilitate statolith growth. Indeed, the fibrous material seen in decalcified mature statoliths may represent a residual matrix material originally present in the CVs. Chapman (1985) believes that the vacuoles of the endodermal cells in the scyphozoan rhopalium may accumulate sulfur by the same pump as exists at the animal's surface...that is, treating the vacuole as external to the cell. In this case, $MgSO_4$ is too soluble to form a precipitate but the highly insoluble $CaSO_4$ would form a precipitate free of magnesium.

Histological Studies. A histological comparison was made between normal ephyrae and those which were statolith-free following development in low sulfate ASW. Whole mounts of ephyrae were treated with toluidine blue, alcian blue, and the modified Hale reaction for detection of acid mucopolysaccharides; the acid Schiff reaction to detect carbohydrates, and Mallory's aniline blue stain to uncover collagen (Spangenberg, 1968). Results of these studies indicated the presence of sulfated acid mucopolysaccharides and of collagenous material in the rhopalia. The statoliths were surrounded by a PAS-positive material which was also positive for collagen using the Mallory's aniline blue technique. Spangenberg (1968) concluded that the presence of sulfated acid mucopolysaccharides and of collagenous material at the site of statolith formation implies that these materials may be involved in statolith synthesis in Aurelia.

In higher organisms, Lyons (1955) related the presence of PAS-positive material in the preotolithic material of the mouse to otolith formation in those animals. She believed that some of this material was mucopolysaccharide in content and pointed out that, in otolith formation, the appearance and development of the mucopolysaccharide parallels that of the calcium salt. According to Lyons, the picture suggested that mucopolysaccharide was important in calcification. Belanger (1953) found that the otolithic membranes of the rat incorporated S^{35} which he believed pointed strongly to the presence of sulfomucopolysaccharides in the otolithic membrane. He reported later (Belanger, 1960) that the otoconia contained both neutral and sulfated mucopolysaccharides and that the hyaluronidase-resistant sulfated mucopolysaccharide was different from the generalized type of chrondroitin sulfate.

Effects of Environmental Factors on Statolith Synthesis

Modified Artificial Sea Water Ingredients. A series of experiments were done to determine the effects of sulfate deficiency, calcium deficiency, and phosphate and strontium addition to ASW on statolith formation in developing ephyrae.

Sulfate Deficiency. Aurelia polyps were induced to strobilate in iodine-containing ASW to the first segment stage (approximately 48 h) and, after rinsing, were transferred to sulfate-free ASW (Spangenberg, 1968). Ephyrae which developed in the sulfate-free ASW were practically statolith free (96.6% had no statoliths). Supplementation of these strobilae developing in sulfate-free ASW with 2 mM of sodium acid phosphate did not alleviate the statolith deficiency in ephyrae which developed from these strobilae.

Histochemical studies were made of rhopalia of the ephyrae which had developed in sulfate-free media and compared with ephyrae which had developed in sulfate-containing ASW. Rhopalia of the sulfate-deprived ephyrae showed less intense staining than controls when treated with the modified Hale reaction at pH 1.5. Also, rhopalia of sulfate-deprived ephyrae stained less intensely than controls using toluidine blue. Alcian blue staining for acid mucopolysaccharides at pH 6.8 and 2.8 failed to demonstrate a difference between the sulfate-deficient and normal ephyrae. This research indicated that, in addition to a need for sulfate to make calcium sulfate statoliths, the organisms also use sulfate to form sulfated acid mucopolysaccharides which may be required for mineralization to occur.

Although statolith formation was not stimulated by phosphate supplementation of sulfate-deprived ephyrae, it was later found that statolith synthesis proceeded when a small amount of sulfate was present in the phosphate supplemented sea water in which strobilae develop (Spangenberg, 1981). Of particular interest was the finding that, whereas 5.6 mM concentration of Na_2SO_4 in ASW was required for significant statolith synthesis, 20 statoliths/rhopalium formed with only 3.5 mM Na_2SO_4 when supplemented with 0.72 mM NaH_2PO_4. Indeed, only 0.7 mM Na_2SO_4 was required for normal statolith synthesis when a higher dosage of 1.44 mM NaH_2PO_4 was used. Phosphate, therefore, was found to be a powerful stimulant for statolith synthesis promoting uptake of calcium as well as sulfur into cells and/or CVs. Energy dispersive X-ray analysis of statoliths of ephyrae supplemented with phosphate, however, failed to detect phosphate in these gypsum statoliths (Spangenberg, 1979). Comparison of statolith synthesis of ephyrae from polyps given ASW with 3.5 mM sulfate plus iodine until the first segment formed and then transferred to ASW with 0.72 mM phosphate until ephyra formation and those given phosphate to the first segment stage and then transferred to ASW containing the sulfate, revealed that statoliths did not form in either case. These results were interpreted to mean that neither sulfate nor phosphate is stored in the organisms during early strobilation for later use in statolith synthesis.

TEM comparisons of rhopalia of ephyrae from

sulfate-deficient media and controls indicated that the calcifying vesicles of the rhopalia of organisms from low sulfate ASW are normal in number, acid phosphatase activity, and in ability to initiate mineralization by forming minute statoliths.

The energy dispersive X-ray analysis studies of rhopalia of sulfate-deficient ephyrae emphasized the fact that calcium as well as sulfate is not detected. That is, calcium is apparently not accumulated and stored in rhopalia in the absence of sulfate. This observation led to the exploration of the effect of low calcium ASW on statolith synthesis. Statolith synthesis proceeds normally with as low as 3.4 mM Ca^{2+} in the ASW during strobilation and ephyra formation. Lower concentrations of calcium in the sea water, however, causes the formation of excessive numbers of small statoliths to form in the rhopalia. Indeed, the average number of statoliths per rhopalium of ephyrae which had developed in ASW containing only 0.7 mM Ca^{2+} was 46.1, nearly double the control number of 24.3. Supplementation of the ASW containing 1.4 mM Ca^{2+} with 0.72 mM sodium acid phosphate led to a statistically significantly higher number of statoliths in the ephyra rhopalia as compared to those not receiving phosphate. Organisms which developed in 0.7 mM Ca^{2+} made very high numbers of statoliths (51-62/rhopalium) with or without the phosphate. On the other hand, organisms which developed in strontium supplemented (0.75 mM) low Ca^{2+} ASW, made significantly higher numbers of statoliths in the 0.7 mM Ca^{2+} containing ASW, but not in the 1.4 mM Ca^{2+} containing ASW. Formation of high numbers of statoliths in these organisms suggested that either more calcifying vesicles are synthesized or more calcifying vesicles are mineralized than in control organisms (Spangenberg, 1979).

The ability of rhopalia to synthesize abnormally high numbers of statoliths in organisms which have severe morphological abnormalities due to calcium deficiency emphasizes that calcium uptake and/or concentration mechanisms are different in rhopalia than in the rest of the organism and it also indicates that rhopalia do not serve as calcium storage sites from which stored calcium is distributed throughout the organism to prevent calcium deficiency effects from occurring. Of particular interest in this study was the finding, through the use of the energy X-ray dispersive analysis, that environmental strontium is incorporated into the statoliths, whereas phosphate is not. Both substances enhanced statolith numbers in low calcium-develop ephyrae but did not alleviate low calcium-induced developmental abnormalities.

X-irradiation. Polyps induced to strobilate with 10^{-5} M thyroxine were X-irradiated with 50, 100, 150 and 200 Gr of X-irradiation. It was found that the lowest dosage tested of 50 Gr significantly reduced statolith numbers in ephyrae developing from the radiated polyps, and, indeed, higher dosages of X-irradiation eliminated statolith formation entirely and caused reduced numbers of rhopalia to form (Prokopchak, et al., 1985). The lack of statoliths in the ephyrae from strobilae which developed after exposure to 150 and 200 Gr of X-rays may have been the result of damage to precursor cells of lithocytes by the X-irradiation.

Clinostat Rotation. Statolith synthesis was also studied in ephyrae which had developed while rotating on a clinostat. The "parent" polyps were induced to strobilate using iodine in ASW while impaled on a cactus spine to maintain them in a fixed position during rotation. It was found that ephyrae which had developed during rotation at 1/2 and 1/4 rpm had statistically significantly fewer statoliths than ephyrae exposed to rotation at 1/15, 1/8, 1, and 24 rpm. It was suggested that the developing ephyrae were disoriented with respect to gravity at speeds of 1/2 and 1/4 rpm, causing fewer lithocytes to differentiate or to mineralize (Spangenberg et al., 1985).

Chemical Pollutant Effects on Statolith Synthesis. During a study to determine the impact of petroleum oil and some of its components on jellyfish development, statolith synthesis was evaluated in ephyrae which had developed in the presence of these pollutants (Spangenberg, 1981).

Table 1. Effects of Alaskan Crude Petroleum Oil (ACPO) and Hydrocarbons on Statolith Synthesis

R = reduced S = small N = normal

Compound	Dosage	Statolith Size	Statolith Numbers
ACPO	0.2%	S	R
Aniline	0.9 mM	N	N
(24 hr transfer)			
Anthracene	2.0 mM	S	N
Benzanthracene	2.0 mM	S	N
Benzene	4.0 mM	S	N
Benzo(α)pyrene	0.2 mM	S	N
Bephinyl	0.02 mM	S	R
Cresol	0.3 mM	S	N
Naphthalene	0.7 mM	N	N
Perylene	2.0 mM	N	N
Phenol	0.5 mM	S	R
Pyrene	2.0 mM	S	R
Toluene	1.0 mM	N	N
Control	---	N	N

Table 1 reveals that ACPO and many of the hydrocarbons tested (anthracene, benzanthracene, benzene, benzo(α)pyrene, biphenyl, cresol, phenol, and pyrene) caused the formation of smaller statoliths than controls. In addition, biphenyl, phenol, pyrene, and ACPO caused a significant reduction in statolith number. Naphthalene, perylene, and toluene did not affect statolith size or number. The reduced size and numbers of statoliths in affected organisms probably represented interference with the uptake and/or concentration of calcium and sulfate at the cellular level. Petroleum and hydrocarbons have also been reported to affect skeletal formation in fish and sea urchins (Struhsaker et al., 1974; Mironov, 1967; DeAngelis and Giordano,

1974; Lonning and Hagstrom, 1975). Statoliths could serve as sensitive indicators which detect environmental pollutants which are deleterious to other mineralizing biological systems.

Effect of Cadmium on Aurelia Statoliths

Statolith Synthesis. An examination of the effects of petroleum oil and related hydrocarbons revealed that statolith synthesis was vulnerable to petroleum oil and some of the hydrocarbons found in the petroleum oil. In addition, petroleum oil contains various trace metals which could also impact on statolith synthesis. One of these is cadmium. Preliminary testing with a dosage range of cadmium administered to polyps at the time of strobilation induction revealed that 4 µM and 5 µM concentrations caused severe abnormalities in developing ephyrae resulting in ephyrae with shortened or no arms. Mortality rate in the 5 µM treated ephyrae was especially high. Emphasis was placed, therefore, on the effects of 1 to 4 µM of cadmium on statolith synthesis. Four tests were done exposing 10 polyps per treatment to 0,1,2,3, and 4 µM cadmium chloride which was added to ASW containing 1 x 10^{-5}M iodine. The tests were done at 30°C. Ten ephyrae collected from each dosage of each test were randomly selected and studied microscopically. Statolith numbers per rhopalium were counted and compared with untreated control ephyrae. The average number of statoliths made per rhopalium per test group of ephyrae was determined. An analysis of variance was done on each series of tests to establish whether the mean of various treatments was significantly different from others of the same test sequence. In all test series, highly significant differences were determined between the groups at the $p < 0.01$ level.

Table 2. Effect of Cadmium on Statolith Formation in Developing Ephrae

Duncan's New Multiple Range Test*

Control	1 µM Cd^{2+}	2 µM Cd^{2+}	3 µM Cd^{2+}	4 µM Cd^{2+}
(n=50)	(n=50)	(n=40)	(n=41)	(n=24)
18.07	17.47	7.92	3.84	3.29

Values underscored are not significantly different. $p = 0.05$.

*Mean number of statoliths per rhopalium

Table 2 shows that statolith numbers were severely reduced in ephyrae that had developed in 2 µM cadmium and the organisms made significantly fewer statoliths (less than one-half) than those ephyrae which had developed in 1 µM cadmium-containing ASW or in non-treated controls. Three and 4 µM of cadmium caused even greater reductions of statolith numbers in ephyrae which had developed in them.

Demineralization. The effects of cadmium on demineralization of statoliths in ephyrae not fed for 8 days after their release from the strobilae were determined. These ephyrae were treated with 3 µM of cadmium in ASW and maintained with untreated control ephyrae at 30°C. Baseline numbers of statoliths representative of the group of ephyrae used for these experiments were determined by counting the number of statoliths in ten of these ephyrae at the beginning of the test period. An average of 28.7 statoliths per rhopalium was found Subsequently, statolith numbers were counted in ten ephyrae from the treated and controls on days 4 and 8 of the experiment. Another group of ephyrae were given 3x and 5x the amount of Ca found in ASW (7.5 µM) in the presence and absence of cadmium. Statistical analyses of the statolith numbers found was done as described for the statolith synthesis studies. Table 3 reveals that cadmium in low dosage accelerated mineral loss from non-fed ephyrae within 8 days of their formation from strobilae.

On day 4 of the experiment, cadmium-treated ephyrae had significantly fewer statoliths than ephyrae in the other groups and cadmium-treated ephyrae supplemented with 3x and 5x calcium in ASW made significantly more statoliths than those given cadmium alone. By the eighth day, however, statolith numbers were considerably reduced in all groups, with the cadmium-treated organisms showing the lowest statolith numbers. In these organisms, the 5x calcium-supplemented-cadmium-treated organisms had significantly more statoliths than the cadmium treated organisms but those given 3x calcium-treated-ASW did not differ significantly from those given cadmium alone. Of greatest importance is the fact that cadmium acts directly in this simple system to cause an increased reduction of statolith numbers and presumably an accelerated rate of calcium loss in the unfed ephyrae.

Table 3. Effect of Cadmium on Statolith Demineralization in Unfed Ephyrae

Duncan's New Multiple Range Test*

Day 4 after Release from Strobila (n = 30)

3 µM Cd^{2+}	3 µM Cd^{2+} + 3 X Ca^{2+}	3 X Ca^{2+}	ASW	5 X Ca^{2+}	3 µM Ca^{2+} + 5 X Ca^{2+}
10.9	12.9	14.3	16.3	17.3	18.1

Day 8 After Release from Strobila

3 µM Cd^{2+}	3 µM Cd^{2+} + 3 X Ca^{2+}	3 µM Cd^{2+} + 5 X Ca^{2+}	3 X Ca^{2+}	5 X Ca^{2+}	ASW
0.43	0.71	2.29	2.3	2.8	3.0

Values underscored are not significantly different, $p = 0.05$

*Mean number of statoliths per rhopalium.

Discussion

Cadmium in low dosages decreased the number of statoliths formed in ephyrae and accelerated mineral loss from non-fed ephyrae within 8 days of their formation from strobilae. Statolith size, however, in these ephyrae which had developed in the presence of cadmium was not reduced. Further, the cadmium-accelerated demineralization of the ephyra statoliths was alleviated significantly by calcium supplementation of the sea water at 5x the levels of the ASW. These findings indicate that cadmium may be affecting calcium uptake into ephyrae during their developmental stages or causing an accelerated loss of calcium during statolith formation. The demineralization results, in fact, indicate that the cadmium acts directly in this simple system to cause an accelerated rate of calcium loss in the unfed ephyrae.

Cadmium toxicity has been associated with mineralization effects in other organisms. Bengtsson et al. (1975) reported a high incidence of vertebral fractures in minnows exposed to sublethal concentrations of cadmium in brackish water. He suggested that cadmium may be causing a demineralization of the skeleton, as reported for human victims of the Itai-Itai disease caused by cadmium toxicity. In flounders, Larsson and Piscator (1971) found reduced levels of potassium, calcium, magnesium, and inorganic phosphate following treatment with sublethal levels of cadmium (0.005-0.500 mg/l). Webb (1979), in an extensive review of the interactions of cadmium with cellular components, suggested that interactions between cadmium and calcium at the cellular level could lead to displacement of calcium from its action sites and a decreased uptake of calcium. Scharpf et al. (1972) demonstrated a dose-dependent delay in fetal skeletal calcification in pregnant rats due to cadmium treatment. Larsson and Piscator (1971) consider that osteomalacia does not result from a direct action of cadmium on bone, but is secondary to renal tubular dysfunction and the failure to reabsorb calcium and phosphorus. Dietary deficiencies of protein and calcium have been shown to aggravate the skeletal changes due to chronic cadmium poisoning in experimental animals and to cause abnormal curvature of the spinal column.

Yoshiki et al. (1975), Kimura and Otaki (1975) and Kawai and Kimura (1975) also concluded that cadmium acts directly on bone, rather than indirectly through a functional change in the kidney. Thus, in rats, on a calcium and Vitamin D deficient diet, the latter authors found cadmium to cause a dose-dependent inhibition of the formation of metaphyseal trabeculae in the tibiae and to reduce the epiphyseal cartilage. At a high level of cadmium in the diet, most of the metaphyseal trabeculae had disappeared after 3 weeks and the epiphyseal cartilage was abnormally narrow, although, at that time there was no histological evidence of kidney damage. Young rats injected with cadmium accumulated the cadmium in osteoblasts (Bawden and Hammarstrom, 1975). A high concentration of cadmium in these cells could interfere with the metabolism of iron, Zn^{2+}, Mn^{2+}, and Cu^{2+}, all of which are necessary for bone formation.

In an earlier study, it was found in our laboratory that thyroxine (2×10^{-5}M) also accelerates the rate of demineralization in unfed ephyrae over an 8 day period. This effect was compared with the osteoporosis caused by thyrotoxicosis in humans and rats (Spangenberg, 1984).

Conclusions

The Cnidaria are composed of numerous organisms with statoliths of either calcium sulfate dihydrate, calcium carbonate, or magnesium calcium phosphate. The Aurelia, which have statocysts on the ends of free swinging rhopalia, are especially suited for statolith research. These organisms are easily reared in the laboratory in the polyp stage, and, when induced to metamorphose with iodine, routinely form statoliths as they develop into the ephyra stage. The statoliths are easily detected microscopically in the living organism and the size and number of statoliths can be readily counted and recorded.

In spite of differences between the formation of calcium sulfate dihydrate statoliths in Aurelia and the formation of calcium carbonate statoliths or calcium phosphate-containing bones and teeth of higher organisms, all of these mineralizing systems have important features in common. Calcium uptake and concentration is essential to most mineralizing systems and cells capable of mineralization must be formed and maintained by these organisms. Many mineralizing systems utilize vesicles such as the calcifying vesicles which are synthesized by jellyfish cells and are retained intracellularly as the cells mineralize. In higher organisms, matrix vesicles are synthesized by cells and extruded into an intracellular matrix where they contribute to the initiation of mineralization (Anderson, 1984; Ascenzi et al., 1982). Sulfated mucopolysaccharides and collagenous materials which are found in the rhopalia of the jellyfish are needed for mineralization in higher organisms. Statolith formation is impaired by chemicals, such as hydrocarbons and cadmium, which also interfere with bone formation and tests of sea urchins. Demineralization of Aurelia statoliths is accelerated by cadmium and thyroxine, agents which reportedly cause demineralization of bone in higher organisms. No doubt continued use of simpler, lower organisms for investigation into mechanisms of mineralization and environmental factors which impact upon them will reveal more similarities between these organisms and higher organisms, and will lead to an improved understanding of mineralization mechanisms in both groups of organisms.

Acknowledgements

The author is grateful to Vera Wang and Suzanne Davis for their technical assistance with the cadmium research. The author's research is

currently funded by NASA, contract #NAG 2-343. The cadmium research was sponsored by DOE, contract #DE-A505-77EV05593.

References

Anderson, HC (1984) Mineralization by matrix vesicles. Scanning Electron Microsc. 1984; II: 953-964.

Ascenzi, A, Bonucci, E, de Barnard, B (Eds.) (1981) Matrix Vesicles. Wichtig Editore, 1-267.

Bawden, FW, Hammerstrom, LE (1975) Distribution of cadmium in developing teeth and bone of young rats. Scand. J. Dent. Res. 83:179-186.

Belanger, LF (1960) Development, structure and composition of the otolithic organs of the rats. In: Calcification in Biological Systems. Sognnaes, RF (Ed.) AAAS:Washington, DC, 151-162.

Belanger, LF (1953) Autoradiographic detection of S^{35} in the membranes of the inner ear of the rat. Science, 118:520-521.

Bengtsson, B, Carlin, CH, Larsson, A, Svanberg, O (1975) High incidence of vertebral damage in minnows, Phoxinus phoxinus, exposed to cadmium. Ambio 4:166-168.

Bigelow, RP (1910) A comparison of the sense organs in medusae of the family Pelagidae, J. Exp. Zool. 9:751-785.

Chapman, DM (1985) X-ray microanalysis of selected coelenterate statoliths, J. Mar. Biol. Assn. U.K. 65:617-627.

DeAngelis, E, Giordano, GG (1974) Sea urchin egg development under the action of benzo(α)pyrene and 7,12-dimethylbenz(α)anthracene, Cancer Res. 34:1275-1280.

Hyman, LH (1940) The Invertebrates. Vol. I, McGraw-Hill Book Company, N.Y., p.505.

Kawai, K, Kimura, M (1975) Renal lesions after a single injection of cadmium in rabbits. Ind. Health. 13:261-265.

Kimura, M, Otaki, N, (1975) Cadmium accumulation in the liver and kidneys of rabbits with single injection. Ind. Health. 13:203-210.

Larsson, S, Piscator, M (1971) Effect of cadmium on skeletal tissue in normal and calcium-deficient rats, Israel J. Med. Sci., 7:495-498.

Lonning, S, Hagstrom, BE (1975) The effects of crude oils and the dispersant Corexit 8666 on sea urchin gametes and embryos, Norw. J. Zool. 23:121-129.

Lyons, MF (1955) The development of the otoliths of the mouse, J. Embryol. Exp. Morph. 3:213-229.

Mironov, OG (1967) The effect of small concentrations of oil and oil products on developing eggs of the Black Sea turbot, Prob. Ichthyol., 7:1-4.

Prokopchak, M, Spangenberg, D, Shaeffer, J (1985) The effects of X-irradiation on the metamorphosis and budding of Aurelia aurita, Amer. Zool. 25:519.

Scharpf, LG, Hill, ID, Wright, PL, Plank, JB, Keplinger, ML, Calandra, JC (1972) Effect of sodium nitrilotriacetate on toxicity, teratogenecity and tissue distribution of cadmium, Nature, 239:231-234.

Singla, CL (1975) Statoliths of hydromedusae, Cell Tiss. Res., 158:392-407.

Spangenberg, DB (1968) Statolith differentiation in Aurelia aurita, J. Exp. Zool. 169:487-500.

Spangenberg, DB (1979) Statolith synthesis and ephyra development in Aurelia metamorphosing in strontium and low calcium containing sea water. Scanning Electron Microsc. 1979;II:433-437.

Spangenberg, DB (1981) The effect of monobasic sodium phosphate on statolith synthesis in Aurelia, Scanning Electron Microsc. 1981; III:355-362.

Spangenberg, DB (1984) Effects of exogenous thyroxine on statolith synthesis and resorption in Aurelia, Amer. Zool. 24:917-923.

Spangenberg, DB (1976) Intracellular statolith synthesis in Aurelia aurita. In: Mechanisms of Mineralization in the Invertebrates and Plants. Watabe, N. and Wilbur, K. (Eds.) U. of South Carolina Press, Columbia,S.C. 377-386.

Spangenberg, DB, Beck, CW (1968) Calcium sulfate dihydrate statoliths in Aurelia, Trans. Amer. Microsc. Soc. 87:329-335.

Spangenberg, DB, Kuenning, W (1976) SEM studies of strobilating Aurelia. In: Coelenterate Ecology and Behavior. Mackie, G.O. (Ed.) Plenum Press, New York, 377-386.

Spangenberg, DB, Davis, S, Ross-Clunis, H (1985) Effects of clinostat rotation on Aurelia statolith synthesis, The Physiologist, 28:S151-S152.

Struhsaker, JW, Eldridge, MB, Echererria, T (1974) Effects of benzene (a water-soluble component of crude oil) on eggs and larvae of Pacific herring and northern anchovy. In: Pollution and Physiology of Marine Organisms, Vernberg FJ, Calabrese A, Thurberg FP, Vernberg WB (eds.) Academic Press, NY,253-284.

Webb, M (1979) Interactions of cadmium with cellular components. In: Webb, M (Ed.) The chemistry, biochemistry, and biology of cadmium, Elsevier North Holland, 285-340.

Yoshiki, S, Yanagisawa, T, Kimura, M, Otari, N, Suzuki, M, Suda, T (1975) Bone and kidney lesion in experimental cadmium intoxication, Arch. Environ. Health., 30:559-562.

Discussion with Reviewers

R.S. Blanquet: What was the purpose or objective of studying rotation on statolith formation since this is not normally experienced by polyps? How were the polyps oriented to the plane of rotation? Why does the author suggest "disorientation at 1/2 and 1/4 rpm but not at higher rpm?"
Author: Polyps were induced to strobilate while rotating on a clinostat to determine whether simulated weightlessness could modify development of ephyrae or their graviceptor structures (rhopalia). While polyps do not experience weightlessness on earth, they are being used for a study of effects of weightlessness, caused by microgravity in outer space, on their development and on their graviceptor structures. The clinostat is used to simulate weightlessness and the polyps were oriented in the horizontal plane on the clinostat while rotating horizontally and in the vertical plane while oriented mouth downwards. (see Spangenberg, et al, 1985). We suggested "disorientation at 1/2 and 1/4 rpm" (but not at other speeds tested) because a biological effect occurred at these speeds during horizontal rotation but not during vertical rotation. Biological effects in other organisms frequently occur at some speeds but not others. Indeed, the speeds at which effects are found provide clues as to the mechanisms involved (i.e. the rate of falling of rhopalia may be modified to a greater degree by some speeds but not others or the sensory cilia may be more stimulated by rotation of the animal at some speeds than other speeds.) The fact that a biological effect occurred at any speed indicates that the synthesis of statoliths may be sensitive to microgravity and should be studied in more detail in space.

R.S. Blanquet: What concentration of hydrocarbon levels were effective on statolith modifications and are these concentrations that would occur naturally after petroleum oil spills or contamination?
Author: The concentration range for the hydrocarbons was from 0.02 mM to 4.0 mM and for the Alaskan crude petroleum oil the range was 0.1-0.3%. These chemicals were added to the artificial sea water at the beginning of the experiment and were not replaced during the 5-6 day testing period. The hydrocarbon dosages may be higher than those found at the site of a petroleum oil spill, but the petroleum oil contains thousands of components. During an oil spill, the effects of these components depend upon the distance the organisms are from the spill and the length of time that the oil is continuously pouring into the ocean, as well as the developmental stages of the organisms exposed. I believe that the statolith sensitivity to hydrocarbons and to petroleum oil could be used as an indicator of calcification effects which occur in other marine organisms, because damage to statoliths would most likely occur earlier than damage to shells of mollusks or bones of fishes. This theory, of course, should be tested by studying jellyfish in nature during an oil spill or by bringing oil-contaminated sea water into the laboratory and testing it on the metamorphosing jellyfish.

R.J. Kingsley: Is it possible that the statoliths of the gastrodermis are not derived from statoliths formed in the epidermis, i.e., each site supports independent development of these structures? Has migration of statoliths been observed? Do you have a proposed route of migration and have intermediate sized statoliths been found between the epidermis and gastrodermis along this route?
Author: In my paper, I state that "the fact that the smallest statoliths are usually found at the proximal end of the statocyst suggests that new statoliths are added to the statocyst in a regulated manner by the migration of cells with tiny intracellular statoliths." Until further studies are done in which the lithocytes with tiny statoliths in the epidermis are labelled in some manner and traced from the gastrodermis to the statocyst, I cannot be certain that these cells migrate nor what route they take. A possible route, however, would be through the mesogloea. Tiny statoliths in calcifying vesicles, however, have not been found in the statocyst, suggesting that early mineralization of statoliths must originate elsewhere and that the mineralizing cells must move into the statocyst.

R.J. Kingsley: How do you interpret the positive acid phosphatase reaction in CVs? Is it possible that there is lysosomal activity occurring in these vesicles? Have lysosomes been specifically identified in areas of statolith calcification?
Author: The CVs could have lysosomal activity. Thyberg and Friberg (1970) reported two types of mineralizing vesicles in the tibial epiphyseal plates of young male guinea pigs. One type (type 1) which had acid phosphatase activity was reported by Thyberg and Friberg to be lysosomes. These authors suggested that lysosomal enzymes may degrade both glycosaminoglycan and the proteins to which they are bound. A local release of the lysosomal enzymes and the chemical changes involved could be of importance in the formation of early mineral deposits, according to these authors. Abolins-Krogis (1973) also found acid phosphatase positive granules in the $CaCo_3$-synthesizing calcifying granules of the snail, Helix pomatia. According to Abolins-Krogis, the enzymes of these granules seem to be engaged in degradation processes necessary for the removal and decomposition of the organic part of the calcifying bodies after mineralization occurs. I have not seen typical lysosomes in the area of statolith calcification, but I believe that the acid-phosphatase-containing CVs could be modified

lysosomes capable of concentrating calcium and sulfate ions for the seeding of statoliths.

R.J. Kingsley: What do you think not feeding ephyrae does to the organisms? The drastic drop in the number of statoliths after eight days suggest to me that the ephyrae may be under severe metabolic stress which could in itself affect statolith formation and demineralization. Is there a normal turnover of mineral in statoliths? If so, does this explain the disappearance of statoliths during starvation, i.e., without food, necessary organic matrix components are missing and those statoliths demineralized in normal turnover cannot be replaced?
Author: The exact mechanisms of the effects of starvation on the jellyfish ephyrae have not been investigated. My interpretation of the effects of starvation on statoliths is that the turnover of calcium is modified so that calcium loss is greater than calcium gain and the calcium is excreted out of the statoliths and into the sea water. The mechanisms have not been studied, but the suggestion that the necessary organic matrix components are lost during starvation, resulting in an inability to replace demineralized statoliths, is a good one and would be a good area to investigate. Of importance, in this regard, is the finding that certain substances (i.e., cadmium and thyroxine) accelerate the rate of statolith loss during starvation, and offer opportunities to investigate their role in demineralization in this relatively simple mineralizing system.

R.J. Kingsley: Do you have thoughts on why, on day 4, there are more statoliths in the 3 µM Cd^{2+} + 5X Ca^{2+} treatment than in the controls?
Author: I do not know why on day 4, there are more statoliths in the 3 M Cd^{2+} + 5X Ca^{2+} than in controls. By Day 8, this group was not different from the controls. Other time periods were not studied.

R.J. Kingsley: Why do you think the 3X and 5X Ca^{2+} treatment yields fewer statoliths than controls? Is it possible that high levels of Ca^{2+} are exerting a toxic effect on the statolith forming cells or metabolism in general?
Author: The 5X Ca^{2+} did not differ significantly from controls on Day 4 or Day 8, but the 3X Ca^{2+} did. If excess calcium were exerting a toxic effect on statolith forming cells or metabolism in general, I would expect the severity of this effect to be higher in the 5X Ca^{2+} groups. In addition, the 5X Ca^{2+} + 3 M Cd^{2+} would then show additive toxic effects rather than a marked improvement of the Cd^{2+}-treated groups, especially as seen on Day 4.

R.J. Kingsley: A decrease in statolith number while maintaining statolith size seems like a real paradox. What are your thoughts? Were observations made on days other than the first, fourth, and eighth?

Author: The lower statolith number but not size statement was in reference to statoliths developing in the presence of cadmium. The lower number probably indicates that fewer cells in the rhopalia mineralized or fewer cells capable of mineralizing differentiated during development of the ephyra. Once the mineralization was initiated, apparently the statoliths were able to grow to a size comparable to those of controls. No differences in statolith sizes as compared to controls were noted in the demineralized organisms. Many statoliths (but not all) of demineralizing controls are found to be smaller than statoliths of newly-formed or fed ephyrae.

R.J. Kingsley: How do you think cadmium is affecting calcium uptake? Is it competing with calcium?
Author: In the case of the jellyfish study, the cadmium may be causing a reduced uptake of calcium or an accelerated loss of calcium in the developing ephyrae. In the nutritionally deprived ephyrae, the cadmium is apparently causing an accelerated loss of calcium. The finding that calcium supplementation causes the ephyrae to retain more statoliths in the presence of Cd^{2+} than in those treated with Cd^{2+} alone indicates that the calcium may have reduced Cd^{2+} uptake into the lithocytes or may simply have provided more calcium to replace that being lost from the lithocytes. Fischer (1985) using cultured Chinese hamster cells, reported that "while magnesium and phosphate ions revealed no effect on Cd^{2+} accumulation, a clear antagonism of Ca^{2+} could be demonstrated." In his discussion, Fischer details other reported interactions between Ca^{2+} and Cd^{2+}. He points out that the ionic radii of Ca^{2+} (0.97 A) and Cd^{2+} (0.99A) are similar, so that perhaps the same uptake processes are used for both metals. Cadmium also inhibits calcium transport in mitochondria (Toury et al., 1985); calcium binding protein calmodulin (Cox and Harrison, 1983); macroscopic calcium currents in rat brain membrane vesicles (Nelson, 1984) by reducing the single channel conductance. Cd^{2+} can also block single cardiac calcium channels as reported in guinea pig ventricular cells by Hess et al. (1984).

R.M. Dillaman: The author states "...that cadmium acts directly in this simple system to cause an increased reduction of statolith numbers and presumably an accelerated rate of calcium loss in the unfed ephyrae." What is your evidence to support such a statement?
Author: My statement that Cd^{2+} acts directly in the simple jellyfish was with regard to the fact that coelenterates do not have organs, such as kidneys or blood systems, which could concentrate Cd^{2+} and cause indirect effects as occurs in higher organisms with complex organ systems.

Additional References

Abolins-Krogis, A, (1973) Fluorescence and histochemical studies of the calcification-initiating lipofuscin type pigment granules in the shell-repair membrane of the snail, Helix pomatia L. Z. Zellfrosh. Mikrosk. Anat. 142:205-221.

Cox, JL, Harrison, Jr. SD, (1983) Correlation of metal toxicity with in vitro calmodulin inhibition. Biochem. Biophys. Res. Comm. 115:106.

Fischer, AB (1985) Factors influencing cadmium uptake and cytotoxicity in cultured cells. Xenobiotica, 8/9:751-757.

Hess, P, Lansman, JB, Tsien, RW (1984) Blockade of single cardiac calcium channels by Mg^{2+}, Cd^{2+} and Ca^{2+} in guinea pig ventricular cells, J. Physiol. (London), 358:61.

Nelson, MT, (1984) Reduction of single calcium channel currents by lanthanum and cadmium. Biophys. J. 45:395A.

Thyberg, J, Friberg, U (1970) Ultrastructure and acid phosphatase activity of matrix vesicles and cytoplasmic dense bodies in the epiphyseal plate. J. Ultrastruct. Res. 33:554-573.

Toury, R, Stelly, N, Boissonneau, E, Dupuis, Y (1985) Degenerative processes in skeletal muscle of Cd^{2+}-treated rats and Cd^{2+} inhibition of mitochondrial Ca^{2+} transport. Toxic. and Appl. Pharm. 77:19-35.